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APPLE SEEDLING RESPONSE TO CALCIUM

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ABSTRACT: Calcium plays an important role in the growth and development of apple trees as well as in high fruit quality. In the present study, solution cultures were carried out under climatically controlled conditions in a growth room to evaluate the response of apple seedlings to six levels of Ca (0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM Ca). Foliar calcium deficiency symptoms were observed at Ca concentrations lower than 0.4 mM. The 3.2 mM Ca treatment resulted in the highest shoot dry matter accumulation and the greatest leaf However, both root length and dry matter area. accumulation were less affected by Ca levels. Calcium uptake parameters were significantly influenced by Ca treatment.

INTRODUCTION

Calcium is essential for the growth and development of apple plants (Malus spp.) and required

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for maintenance of fruit quality. Fruit physiological disorders, such as bitter pit, are associated with low levels of fruit Ca. Calcium movement in the tree needs to be understood before making an evaluation of Ca translocation into the fruit. It has been reported that Ca uptake changes according to changes in Ca concentration in the substrate (1). Foliar symptoms of Ca deficiency in fruit trees have been observed in orchards and under controlled conditions (2, 3, 4, 5). Symptoms develop first on young rapidly metabolizing meristematic terminals (6). Development of fine roots and root hairs is adversely affected by Ca deficiency. This study was designed to evaluate 'York' apple (Malus domestica) seedlings to evaluate shoot and root growth responses, mineral uptake, and mineral efficiency ratios when subjected to various levels of Ca in nutrient solutions.

MATERIALS AND METHODS

<u>Plant Growth Conditions</u>: The experiment was conducted in a controlled environment room with 14 hours of light at 550 μ mole s⁻¹m⁻² light, 8 hours of darkness and one hour of 55 μ mole s⁻¹ m⁻² light preceding and following the light cycle. Daytime temperature was 28±1C with 60% relative humidity and nighttime temperature was 22±1C with 80% relative humidity.

Apple seeds were germinated and grown in flats of sand with a weekly addition of 30 ml of nutrient solution containing (in mM) 3.50 NO₃-N, 0.88 P, 2.15 K, 1.10 Mg, 2.54 Ca, 0.53 S and (in μ M) 23.45 B, 2.96 Mn, 3.83 Zn, 0.80 Cu, 0.29 Mo and 89.25 Fe adjusted to pH of 5.8. Sufficient deionized water was added to maintain water availability near 33 kPa tension. After 60 days of growth, seedlings were removed and transferred to solution cultures containing the

previousle described nutrient composition. seedlings were selected after 10 days of growth in nutrient solution.

Roots were rinsed in deionized water and one seedling was suspended in a 1.9 L polyethylene pot fitted with high density styrofoam tops. The nutrient solution composition was (in mM) 3.5 NO₃-N, 0.5 P, 2.0 K, 1.5 Mg, 0.5 S and (in μ M) 46 B, 9 Mn, 0.8 Zn, 0.3 Cu. 0.5 Mo and 40 Fe. Iron was added as Fe-EDDHA o [ferrous-N,N-ethylenebis(2,(2-hydroxyphenyl))]. levels of Ca (0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM), as $^{rac{G}{2}}$ CaCl,, were established and solution pH was adjusted to 5.8. Solution pH was maintained by adding 1N HCl or 1M $\ddot{\vec{a}}$ KOH every other day. Loss of water from the pot was compensated by addition of deionized water.

After a 35 day exposure period the roots were g rinsed in 5 mM MgCl₂ for 30 seconds followed by a ੀ thorough rinse in deionized water. Root radius (R) was measured using a binocular microscope and root length (L) was measured with a Comair Root Length Scanner. Plant tops were washed in deionized water and then separated into land separated into leaf and stem samples. Leaf area (LA) mas measured using a Licor leaf area meter equipped with a LI-3050 Transparent Belt Conveyor Accessory.

Root and top samples were oven-dried at 70C for three days and dry weight of shoot, leaf, and root Tissue samples were ground and digested in a HNO3: HC103 (4:1) mixture. Element concentrations were determined by inductively coupled plasma emission spectroscopy.

Plant Growth Parameters:

Root surface area (RSA, cm2 plant-1) was calculated as:

RSA = (2) (R) (L)

where: R = root radius (cm)

 $L = root length (cm plant^{-1})$

Root length per gram of root (RL2, m g-root-1) was calculated as:

RL2 = (m root length plant⁻¹)/(g root plant⁻¹)
The shoot efficiency ratio for Ca (ERS) was
calculated as:

ERS = (mg dry shoot wt)/(mg of Ca in shoot) Nutrient influx (IN, pmoles cm⁻¹ s⁻¹) was calculated as:

IN = $[(U_2 - U_1)/(T_2 - T_1)][(lnL_2 - lnL_1)/(L_2 - L_1)]$ where: U = ion content (moles plant⁻¹)

T = time in seconds

L = root length (cm plant⁻¹)

1 & 2 = refer to planting & harvesting times, respectively

Root absorption power (RAP, cm s⁻¹ 10⁻⁴) was calculated as:

RAP = [(IN)/2 RC)]

where: C = mean ion concentration (mol cm⁻³)

R = root radius (cm)

Statistical Analysis: All growth and nutrient uptake parameters were statistically analyzed by SAS programs (7). The simple correlation coefficients (r values) between various plant parameters were also determined.

RESULTS and DISCUSSION

<u>Visual Observations</u>: Calcium levels markedly affected the visual growth of the apple seedlings. Shear (6) has reported that young rapidly metabolizing terminal growth was the first vegetative tissue to display Ca deficiency symptoms. In the present study, similar symptoms were observed at 0.1 mM Ca (Fig. 1A). The effect of Ca deficiency on foliar symptoms, chlorosis to necrosis, is depicted in Fig 1B.

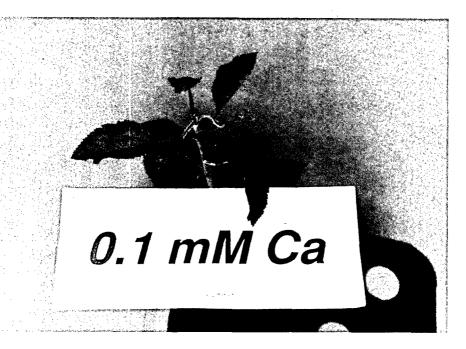


Figure 1a. The expression of calcium deficiency symptoms in 'York' apple seedlings grown in solution culture: (A) Ca deficient shoot and (B) Ca deficient leaves'

Visual observations on the root systems qualitatively indicated a more massive root mass with increasing Ca.

Shoot and Root Growth: Shoot and root parameters as affected by Ca levels are presented in Table 1. Leaf area (LA) was markedly increased at the 3.2 mM Ca level compared to Ca levels of 0.4 mM and below. Root length (L), radius (R), surface area (RSA), and root length per gram of root (RL2) were significantly affected by Ca levels. Higher R and RSA values were observed at high Ca levels (1.6 and 3.2 mM Ca). These results

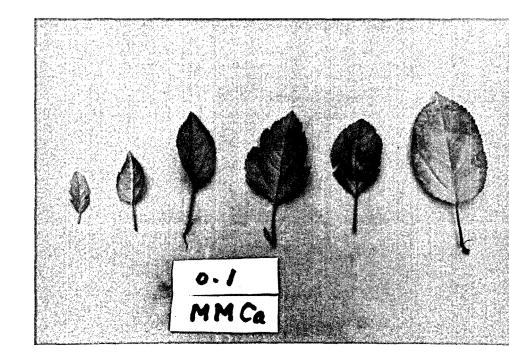


Figure 1b.

indicate that Ca, at the higher levels, resulted in enhanced plant structure. The positive increase in leaf area and root growth agree with previous reports (8).

Stem dry matter was not significantly different among Ca treatments (Table 1) and both leaf and root dry matter production were similarly not significantly different among Ca treatments (data not shown). Shoot:root ratio was not affected by Ca levels mainly because shoot and root dry matter was not affected. Our results indicate that dry matter of 'York' apple

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levels.		
Table 1. Shoot and root growth parameters of 'York' apple seedling at various Ca levels.		2
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ر د د د د د د د د د د د د د د د د د د د	SDW	ΓΑ	ىـ	œ	RSA	RL2
I E E E	g planit	cm² plant"	m plant-1	E	cm² plant-1	m g root-1
0.1	1.12	104	13.9	0.20	172	40.9
0.2	1.02	108	17.5	0.19	203	58.5
0.4	99.0	76	10.2	0.19	122	57.5
0.8	1.04	126	13.2	0.20	165	80.2
1.6	1.10	124	14.4	0.32	291	59.3
3.2	1.14	166	16.5	0.29	301	48.7
rsD**	0.70	74	8.9	90.0	106	26.3

* SDW = shoot dry weight; LA = leaf area; L = root length; R = root radius; RSA = root surface area; RL2 = root length per gram of root.
** LSD = least significant difference, 5% level.

Ca level mM	Shoot			Root			
	Ca mg g ⁻¹	Mg mg g-1	μg g-z	Fe μg g-1	Ca mg g-1	Mg mg g-1	Fe μg g−4
0.1	5.09	8.11	72	142	1.77	3.89	421
0.2	4.88	6.52	40	154	1.66	3.88	447
0.4	6.28	7.21	61	261	1.78	2.33	573
0.8	6.66	5.31	47	109	2.65	2.18	844
1.6	10.33	5.37	52	138	2.27	1.95	440
3.2	8.28	3.34	42	159	3.22	1.66	384
LSD*	2.94	1.80	28	103	1.56	1.75	349

^{*} LSD = least significant difference, at 5% level.

seedlings was not significantly affected by Ca treatment level, under a short-term Ca deficiency, even though Ca is an essential element.

<u>Elemental Concentrations</u>: Shoot (leaf and stems) and root elemental concentrations which were significantly different among Ca treatments are shown in Table 2. Calcium concentrations of shoot increased with increasing Ca levels. Surplus Ca, absorbed by the root, is apparently translocated to the shoot (8).

Magnesium is known to exert an inhibiting effect on the uptake and translocation of Ca in plants (9).

Magnesium concentration in shoots and roots was significantly higher at lower Ca levels than that observed at higher Ca levels. Hogue (9) has reported an interference in Mg uptake due to Ca, which is similar to the current results.

Root Mn concentration was not affected by Ca (data not shown), however, higher shoot Mn concentrations were observed at lower Ca levels (Table 2). This suggests that Ca might interfere with Mn, particularly in Mn translocation to shoots.

Concentrations of Fe in shoot and root were significantly affected by Ca levels, but higher concentrations of Fe were found with the mid-range of Ca levels. This suggests existence of a Ca/Fe imbalance at low and high available Ca levels. Zinc concentrations in shoot and root were not affected by Ca levels (data not shown).

Calcium Uptake Parameters: Increasing Ca levels significantly increased Ca translocation to the shoot, US (Table 3). With the exception of root uptake of Ca at 3.2 mM Ca levels, the uptake of Ca by roots (UR) was similar.

Efficiency ratios for Ca (ERS) declined as the Ca levels increased in the growth medium, indicating that

Table 3. Calcium uptake parameters of 'York' apple seedlings at various Ca levels.

Ca level			Ca Uptake Parameter*		
mM 	us	UR	ERS	IN	RAP
0.1	67.5	14.9	217	47.7	50.2
0.2	61.1	12.4	208	31.6	18.8
0.4	51.2	9.4	116	35.8	10.6
0.8	90.0	11.2	151	55.0	7.4
1.6	138.7	11.3	129	82.3	3.4
3.2	132.4	28.2	100	72.1	1.8
LSD**	79.6	13.2	71	48.6	17.5

^{*} US =- uptake by shoot, mmoles plant⁻¹; UR = uptake by root, mmoles plant⁻¹; ERS = shoot efficiency ratio,(mg of dry shoot)(mg Ca in shoot); IN = Ca influx, pmoles cm⁻¹ s⁻¹; RAP = root absorption power, cm s⁻¹ x 10⁻⁴.

** LSD = least significant difference, 5% level.

at low Ca availability, 'York' apple seedlings are efficient in utilization of absorbed Ca.

Higher Ca influx (IN) values were observed at higher Ca levels. However, significantly higher root absorption power for Ca (RAP) was observed with 0.1 mM Ca and increasing Ca levels in the growth medium reduced the magnitude of RAP for Ca.

CONCLUSIONS

Calcium exerts a strong influence on apple growth with massive, brown-colored root systems developing at the highest (3.2 mM) Ca treatment. Although both root and shoot growth parameters were enhanced with Ca, plant dry matter was not significantly affected by Ca treatments. The highest RAP was observed at 0.1mM Ca, while the highest UR occurred at 3.2mM Ca. There was a general increase in IN with increasing supply of Ca. Apple trees efficiently utilize Ca from dilute solutions. Magnesium, Mn, and Fe foliar concentrations were all lowered with increasing solution Ca.

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